Recombinant DNA Technology

What is Recombinant DNA Technology?

- **Recombinant DNA technology** is a technology which allows DNA to be produced via artificial means.
- The procedure has been used to change DNA in living organisms and may have even more practical uses in the future.
- It is an area of medical science that is **just beginning** to be researched in a concerted effort.

- DNA molecules that are constructed with DNA from different sources are called recombinant DNA molecules.
- Recombinant DNA molecules are created in nature more often than in the laboratory;
  - for example, every time a bacteria phage or eukaryotic virus infects its host cell and integrates its DNA into the host genome, a recombinant is created.
  - Occasionally, these viruses pick up a fragment of host DNA when they excise from their host's genome; these naturally occurring recombinant DNA molecules have been used to study some genes.

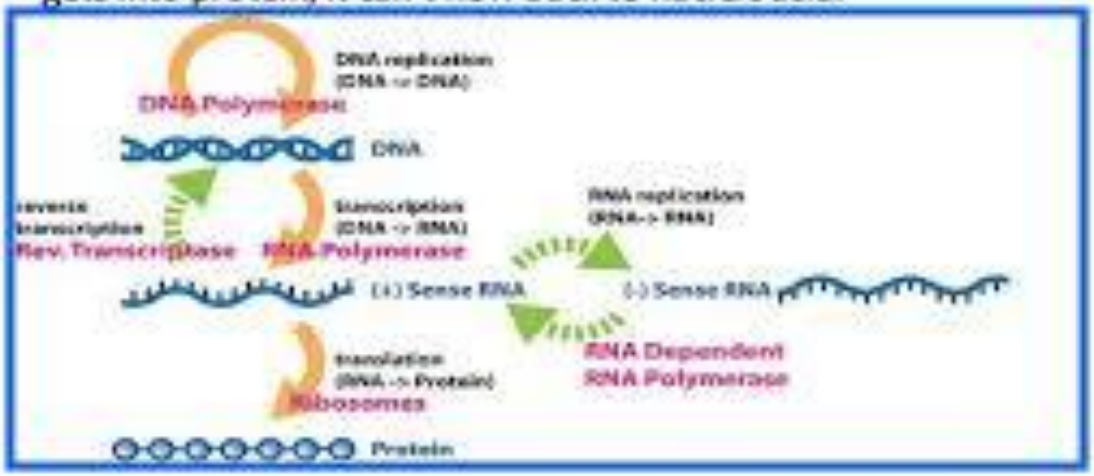
- **Recombinant DNA technology——A series of procedures used to join together (recombine) DNA segments. A recombinant DNA molecule is constructed (recombined) from segments from 2 or more different DNA molecules. Under certain conditions, a recombinant DNA molecule can enter a cell and replicate there, autonomously (on its own) or after it has become integrated into a chromosome.**

Basic principles of rDNA technology:

- Generation of DNA fragments & selection of the desired piece of DNA.
- Insertion of the selected DNA into a cloning vector to create a rDNA or chimeric DNA.
- Introduction of the recombinant vectors into host cells.
- Multiplication & selection of clones containing the recombinant molecules.
- Expression of the gene to produce the desired product.

Central Dogma of Molecular Biology:

- The flow of genetic information in the cell starts at DNA, which replicates to form more DNA.
- Information is then 'transcribed" into RNA, and then it is "translated" into protein.
- The proteins do most of the work in the cell. Once information gets into protein, it can't flow back to nucleic acid.



History of recombinant DNA technology

- Recombinant DNA technology is one of the recent advances in biotechnology, which was developed by two scientists named Boyer and Cohen in 1973.

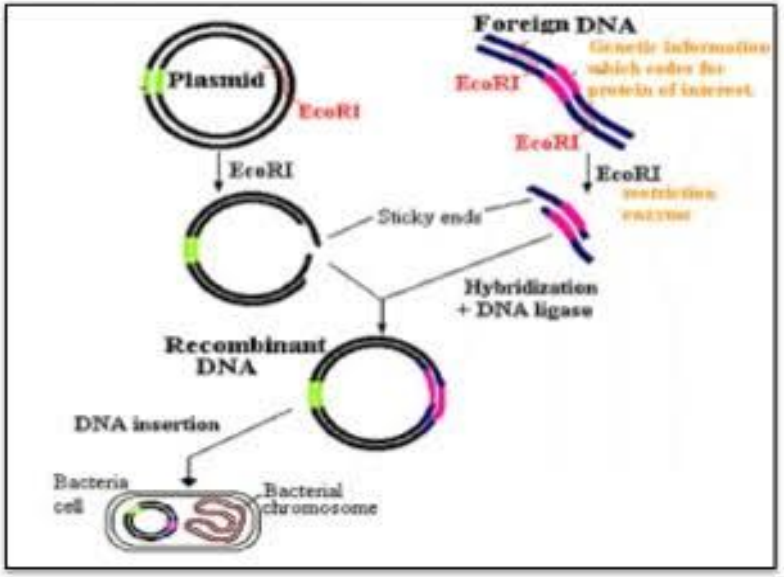
RECOMBINANT DNA (r DNA)

DNA that has been created artificially (not natural). DNA from two or more sources is incorporated into a single recombinant molecule

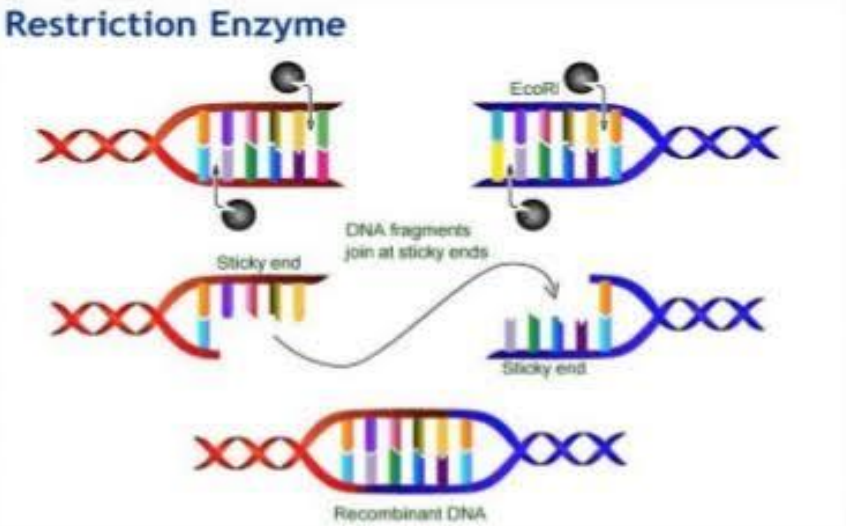
Recombinant DNA(rDNA) is a form of artificial DNA that is created by combining two or more sequences. It is made possible by two important enzymes. **Restriction enzymes** and **DNA Ligase** are the two principal tools, first used by Paul Berg in 1972,employed to alter DNA.

❑ **METHODS BY WHICH RECOMBINANT DNA IS MADE ARE:**

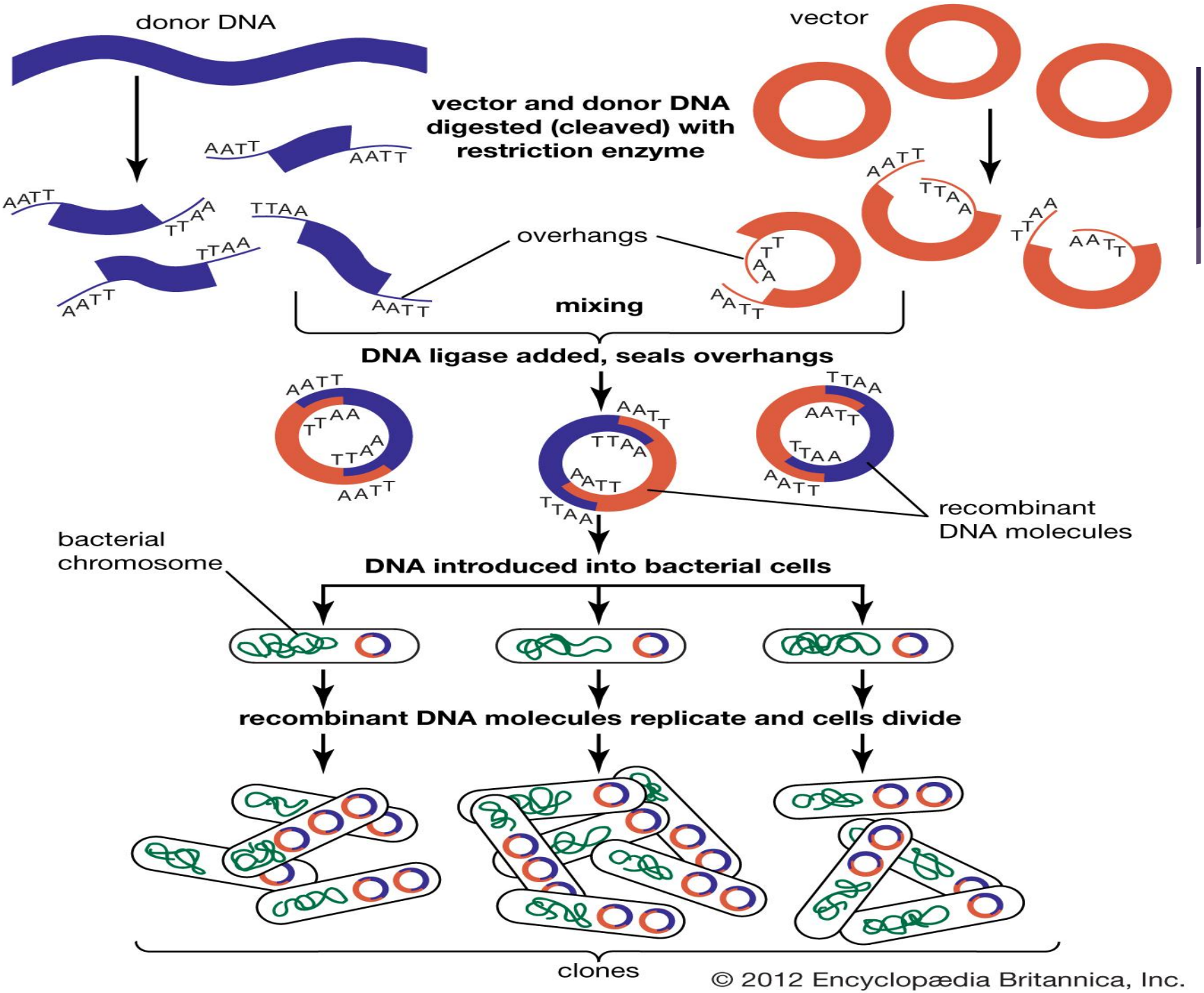
- TRANSFORMATION
- TRANSDUCTION
- CONJUGATION



Recombinant DNA technology



Molecular biology
Second semester
4-2-2013



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1. Clone a gene of interest into plasmid transfer vector

Plasmid transfer vector

+

Gene of interest

=

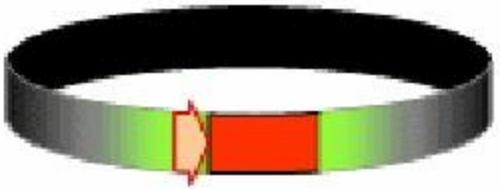
Recombinant plasmid vector

2. Co-transfect the recombinant vector and linearized baculovirus DNA into insect cells

+

Linearized baculovirus DNA

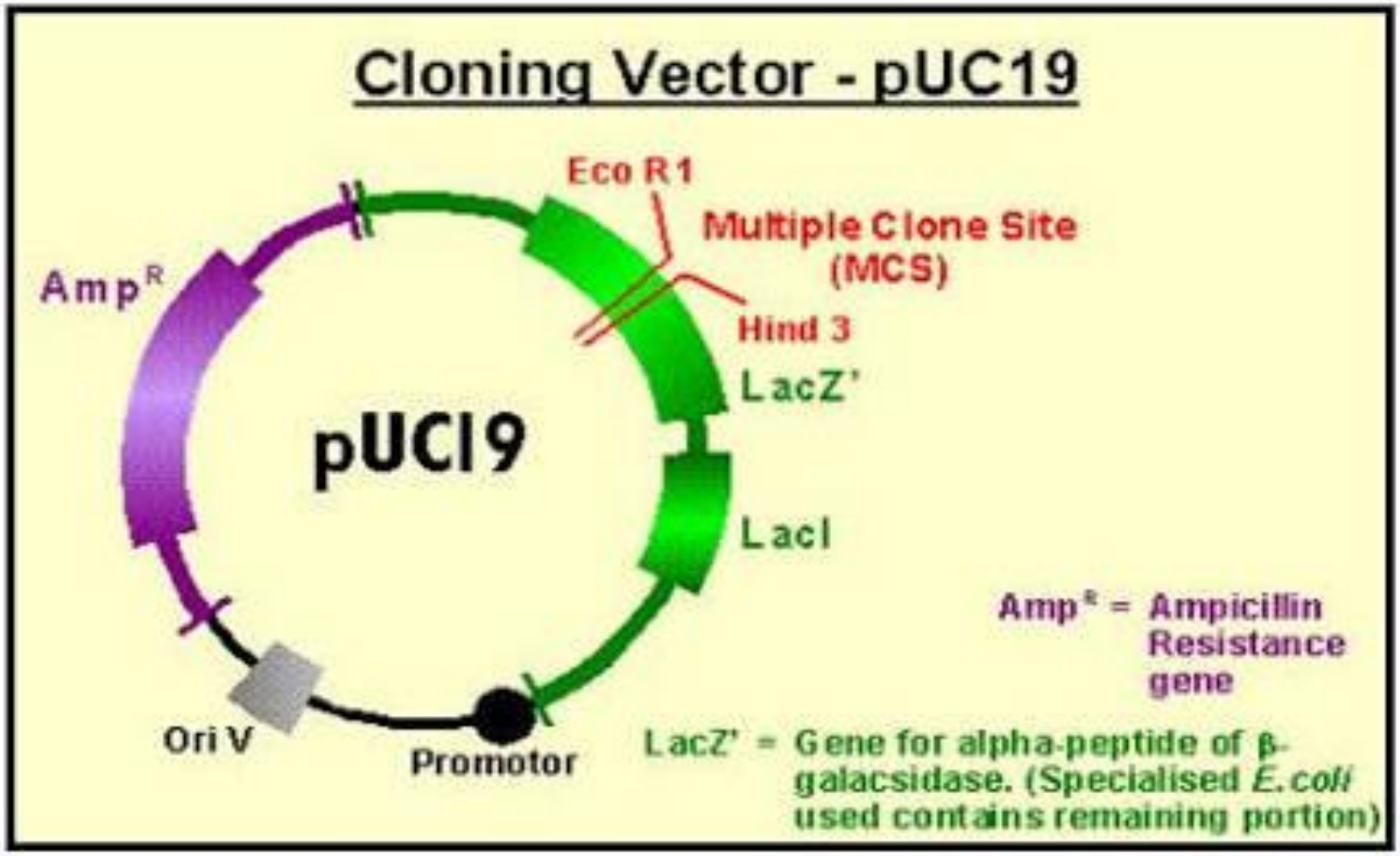
not infectious



Circular recombinant baculovirus DNA

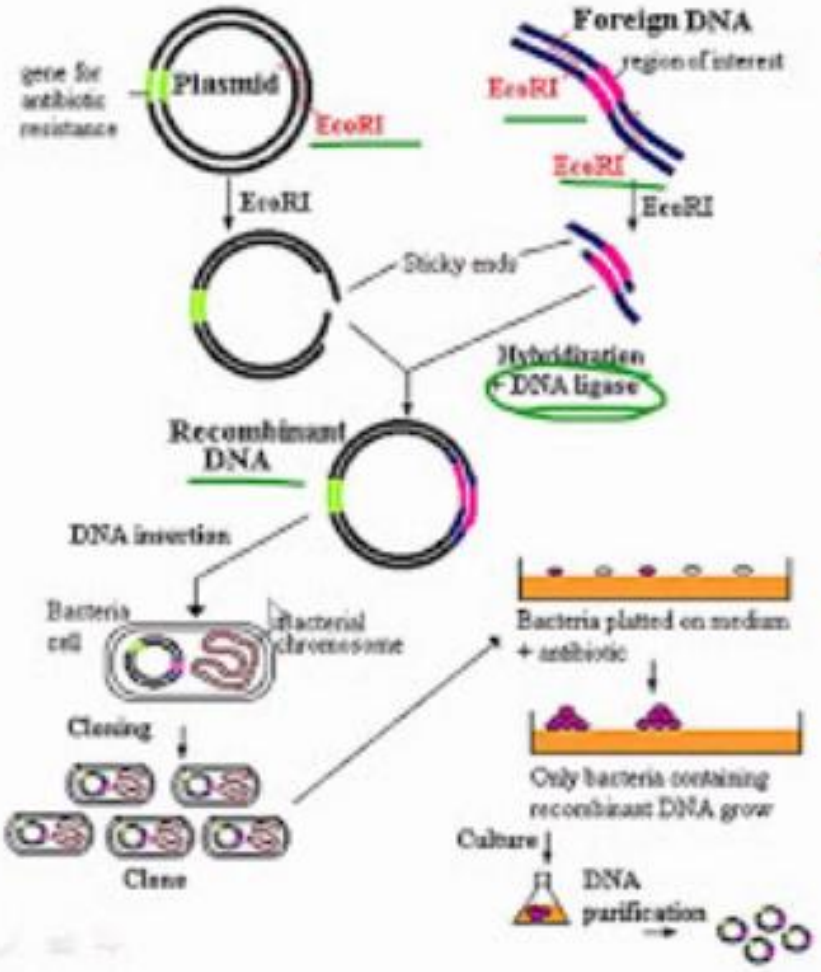
infectious

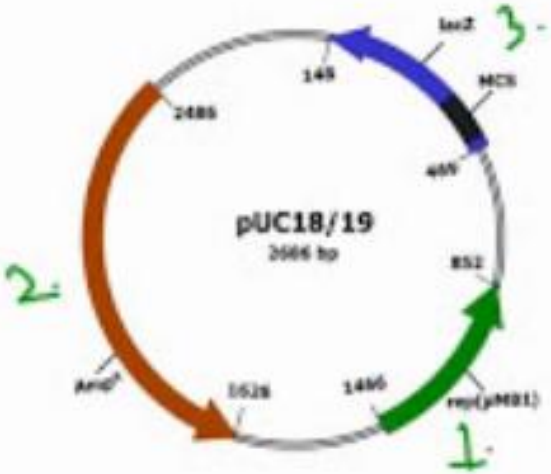
Recombinant baculovirus



Cloning vector

1 · Plasmid vectors





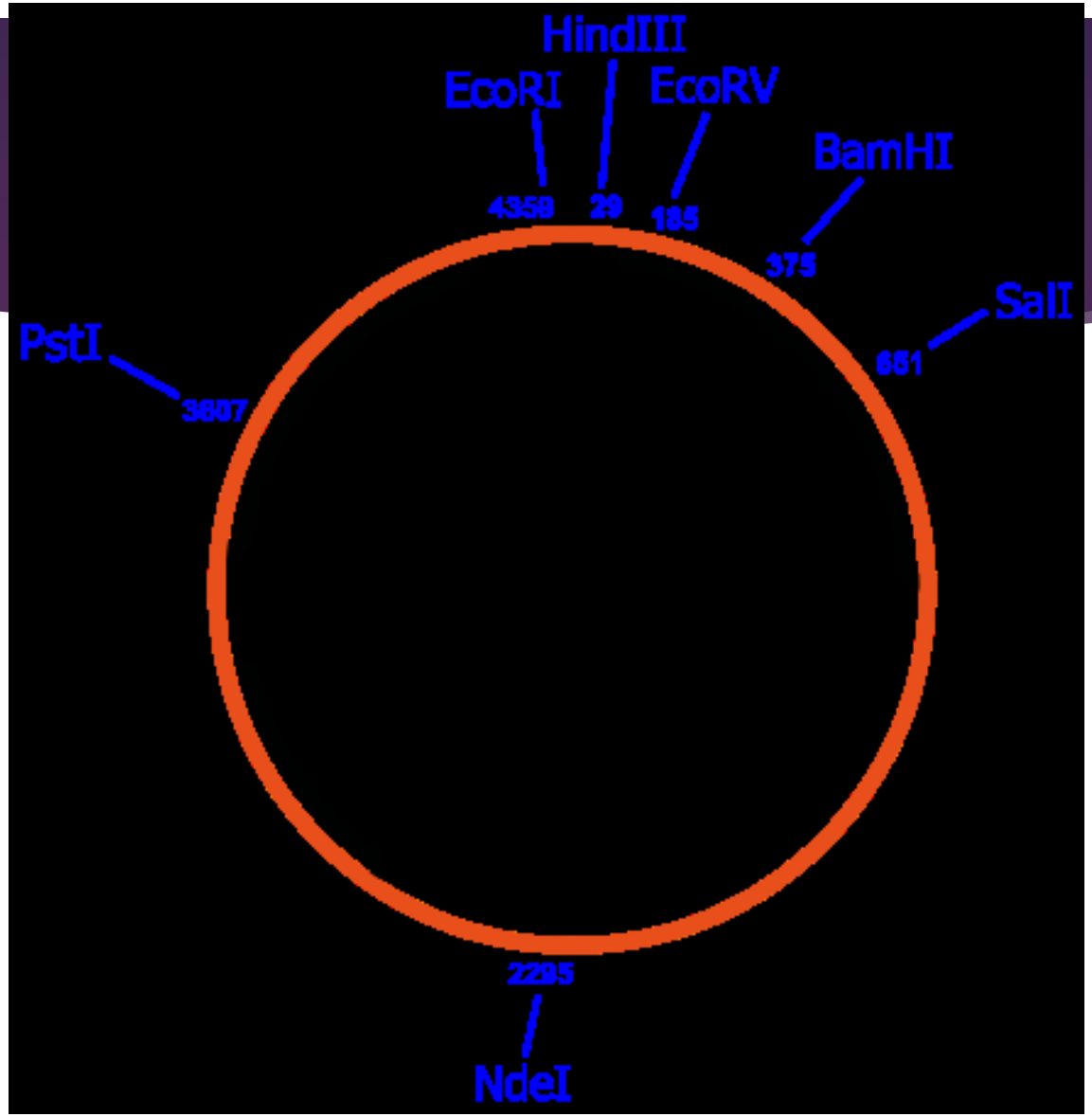
small size

high copy number (number of plasmids maintained per cell)

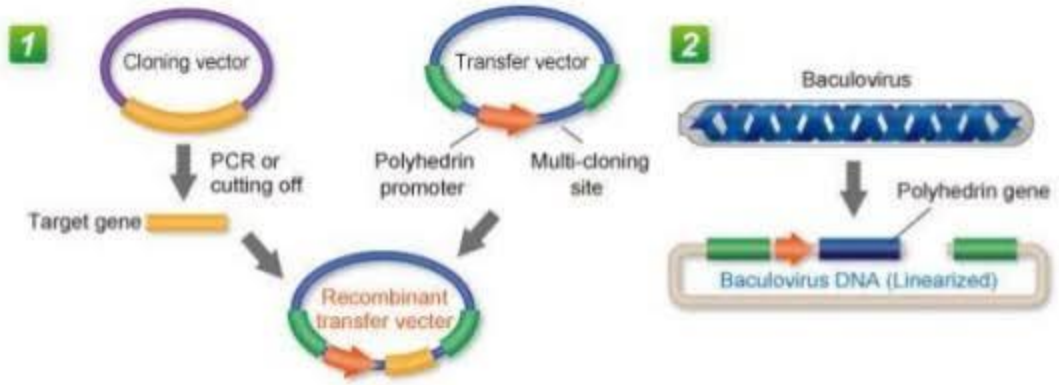
marker gene(s) for easy selection in bacteria or other hosts

visual marker for selecting bacteria carrying plasmids with inserts (blue white screening)

promoters for *in vitro* transcription


HindIII
EcoRI
EcoRV
BamHI
4359
29
185
375
SalI
651
PstI
3807
2285
NdeI

Homologous Recombination

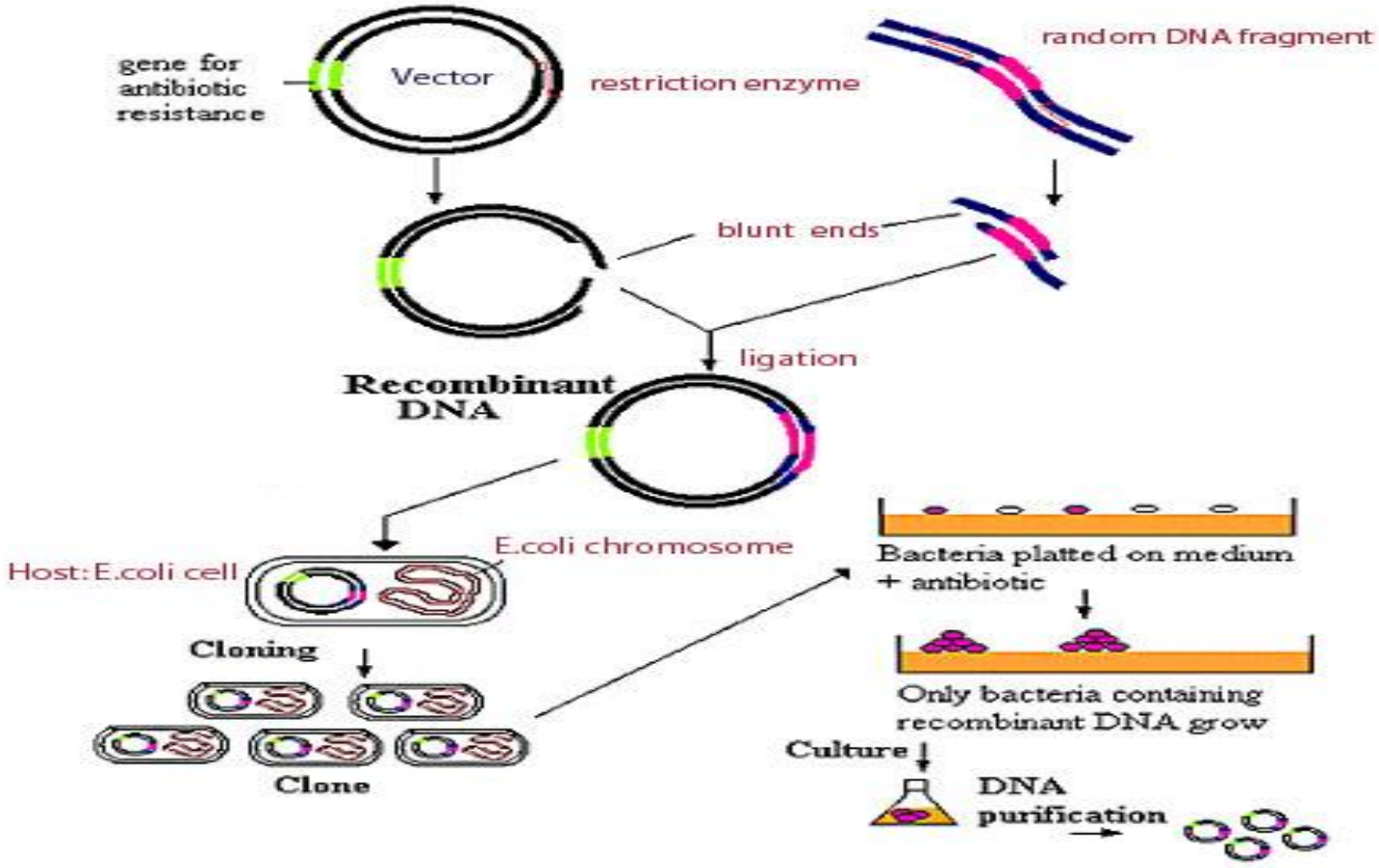


•Transfer vector – **pVL 1393, pVL1392 (9.8kb)**

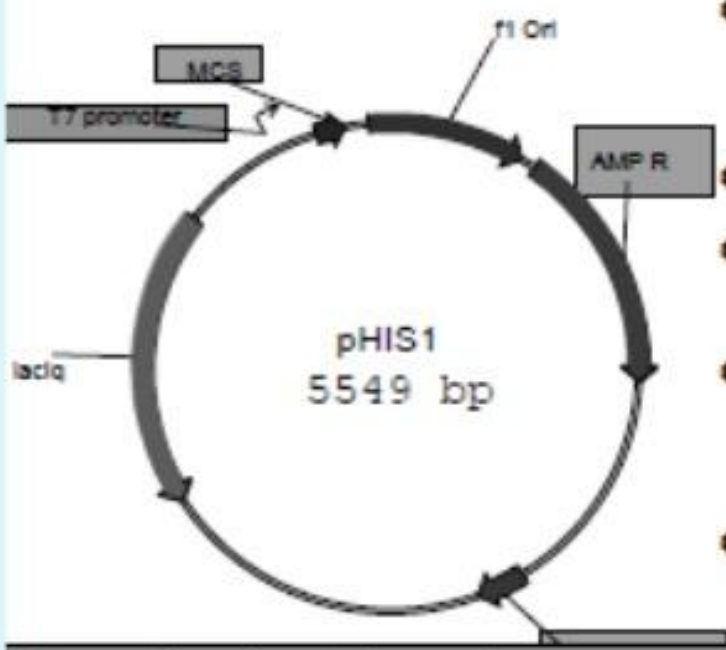
Features

•Multiple cloning sites
•Recombination sequences for insetion in to the Baculovirus genome
•Ployhedrin enhancer- promoter
•Ampiciline resistance gene for selection

Cloning into the vector



Expression vector



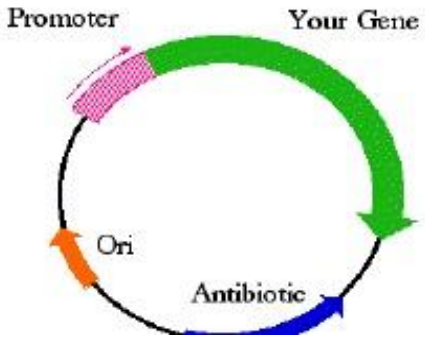
- Ampicillin resistance marker
- ColE1 origin of replication (ori)
- f1 origin of replication (allows single stranded vector to be produced when co-infected with M13 helper phage)
- *lacI* gene (lac repressor protein)
- T7 promoter (specific for phage T7 **RNA polymerase**)
- multiple cloning site (polylinker region) downstream of the T7 promoter
- 6 Histidine tag- useful for purification of protein by Metal affinity chromatography

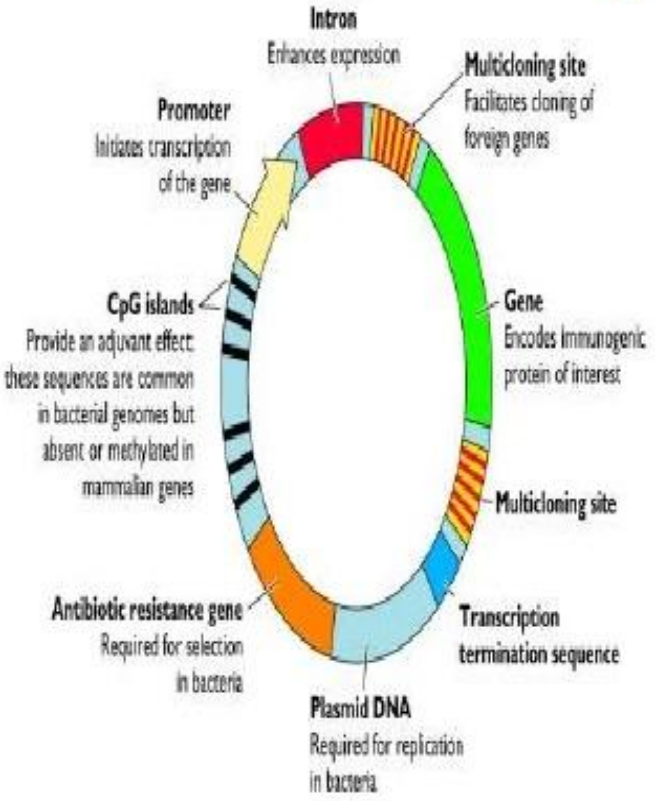
Recombinant protein can be expressed only in *Ecoli* host which can produce T7 RNA polymerase (BL21DE3)

vage site

EXPRESSION VECTORS

- It is engineered plasmid to contain regulatory sequences that act as enhancer and promoter regions and lead to efficient transcription of the gene carried on the expression vector.
- Goal -is the production of large amounts of stable messenger RNA, and therefore proteins
  - **Origin of replication**: DNA polymerase
  - **selectable marker(s):** antibiotic resistance
  - **promoter:** recognized by RNA polymerase
  - **multiple cloning sites** (restriction enzyme sites): cutting/pasting of DNA fragments.
  - **Termination site:** to stop the transcription.

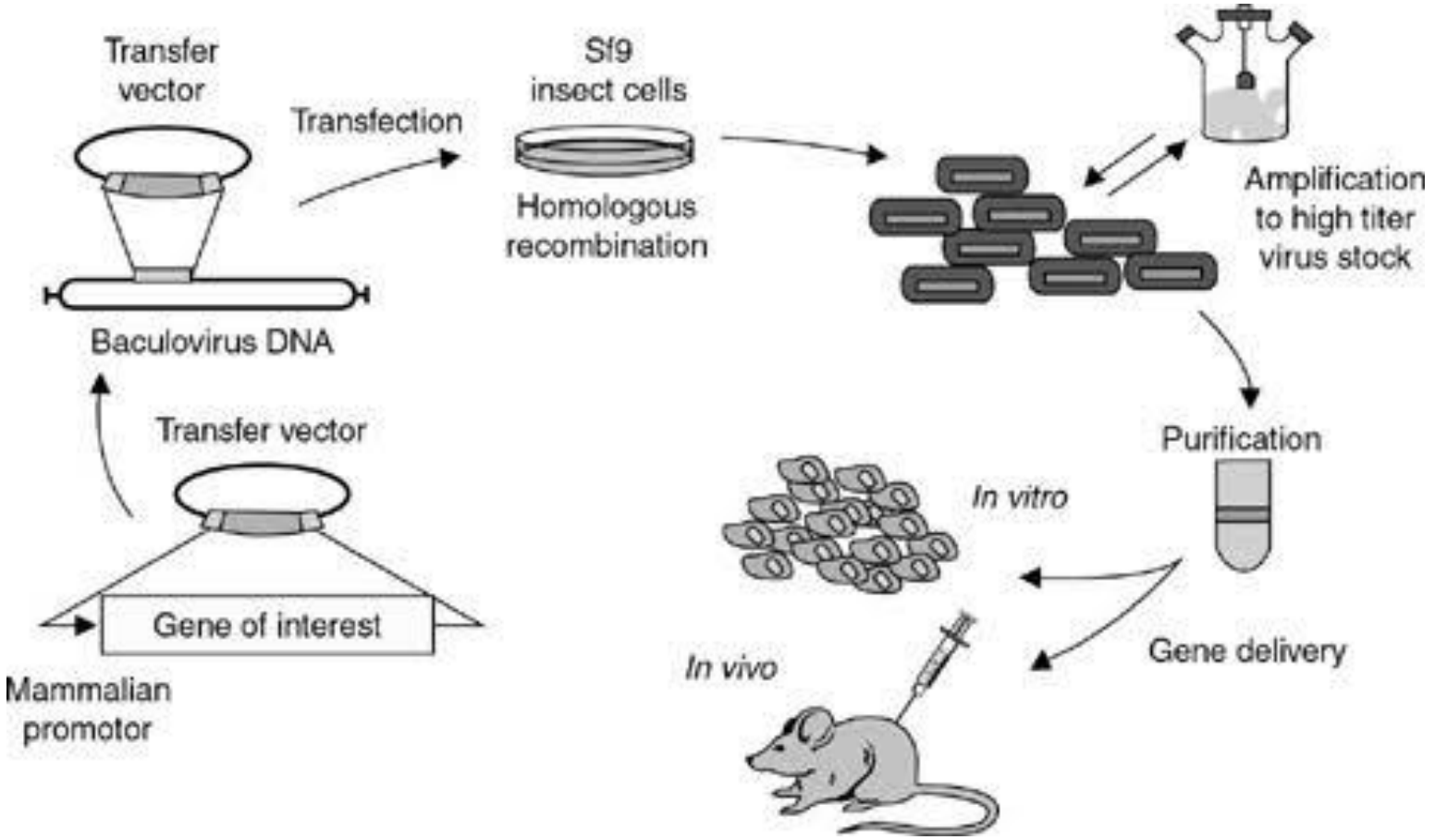




Adapted from M. Oyaski and H. Eril, Sci. Med. 7:30–39, 2000, with permission.

APPLICATIONS OF RECOMBINANT DNA TECHNOLOGY

- Recombinant DNA technology has a wide range of application in industries, medical science, and agriculture as "well as molecular biology.
- Molecular diagnosis of diseases
- Gene therapy
- DNA finger printing
- Production of vaccines
- Commercial and pharmaceutical products

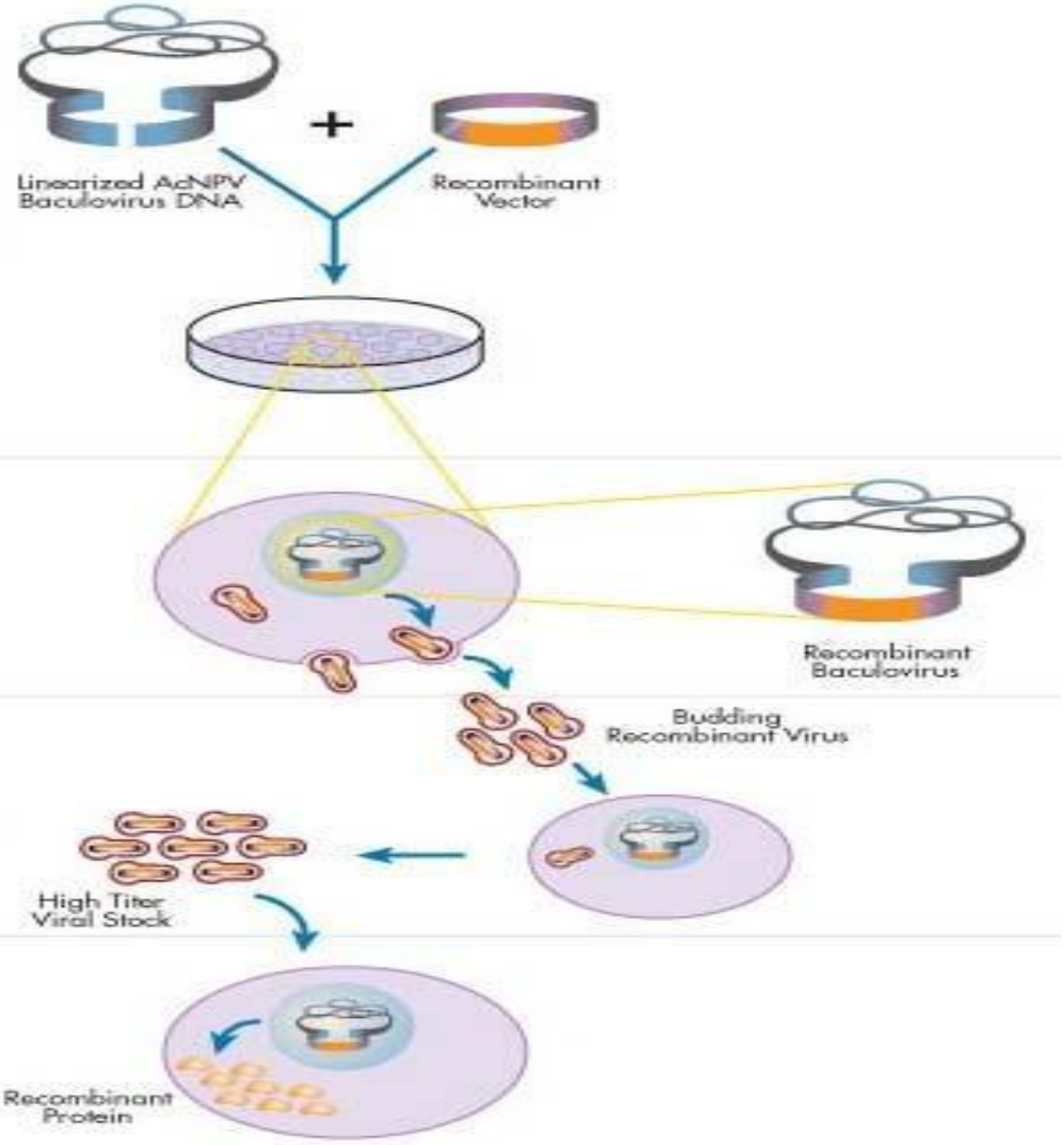

Transfer vector
Transfection
Sf9 insect cells
Homologous recombination
Amplification to high titer virus stock
Baculovirus DNA
Transfer vector
Purification
In vitro
Gene of interest
Mammalian promotor
Gene delivery
In vivo

Co-transfect BaculoGold™ and recombinant transfer vector into *Sf* insect cells

Recombination between the vector and the viral DNA occurs within the cell and recombinant baculovirus are produced

Harvest recombinant virus and amplify to produce high titer stock

Infect cells for recombinant protein expression and purification



PROPERTIES OF A GOOD Recombinant VACCINE

- Ability to elicit the appropriate immune response for a particular pathogen.
- Long term protection
- Safety
- Stability
- inexpensiveness

RECOMBINANT VACCINE

- A recombinant vaccine is a vaccine is produced by r-DNA technology.
- This involves inserting the DNA encoding an antigen that stimulates an immune response into mammalian cells or bacterial cells ;expressing the antigen in these cells and purifying it from them.
- First recombinant vaccine –Hepatitis B vaccine

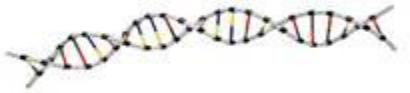
Types of Recombinant vaccines

- Subunit recombinant vaccines – includes proteins, peptides and dna
- Attenuated recombinant vaccines –these genetically modified pathogenic organism(bact. , virus) that are made of non pathogenic and use as vaccines.
- Vector recombinant vaccines- these are genetically modified viral vectors that van be used as vaccines against certain pathogens.

Processing of recombinant DNA Technology

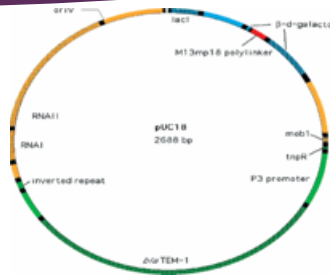
- Isolation of the genetic material
- Cutting of DNA at Specific location
- Amplification of Gene of interest using PCR
- Inserting of Recombinant DNA into the Host cell/organism
- Obtaining the foreign gene product

HOW DNA VACCINE IS MADE

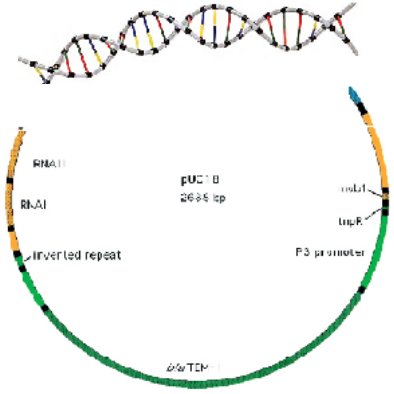


Viral gene

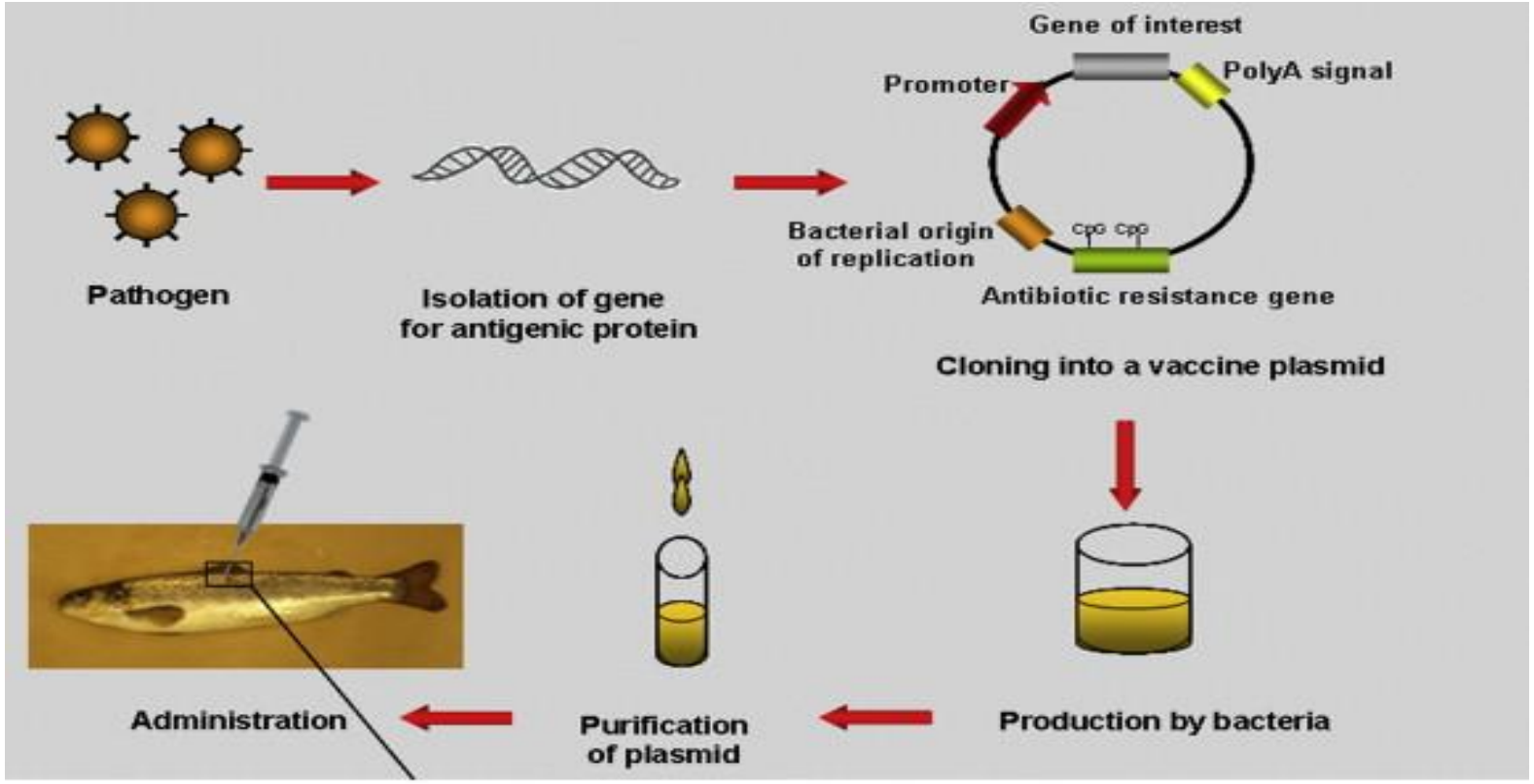
Recombinant DNA Technology



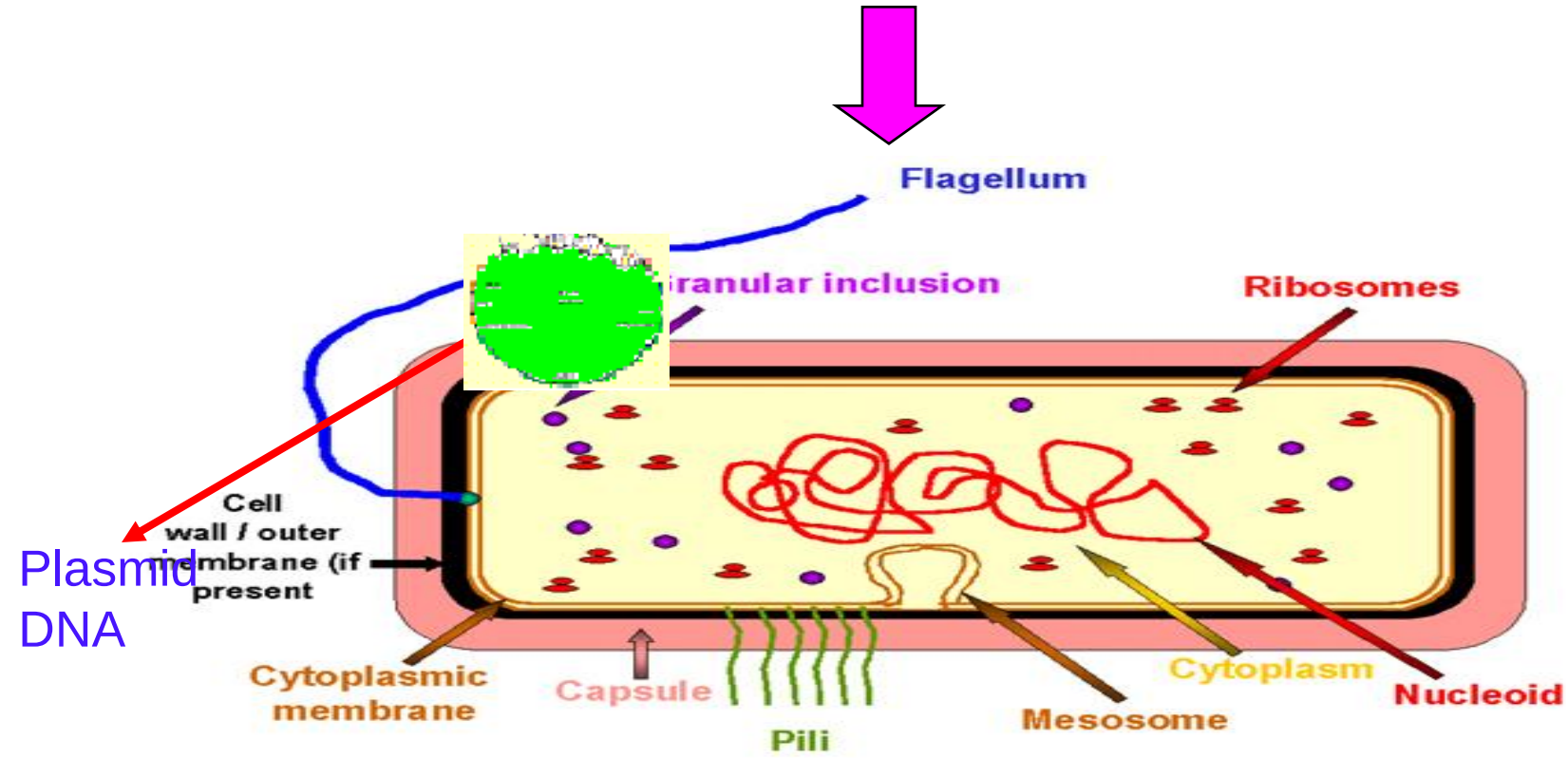
Expression plasmid



Plasmid with foreign gene

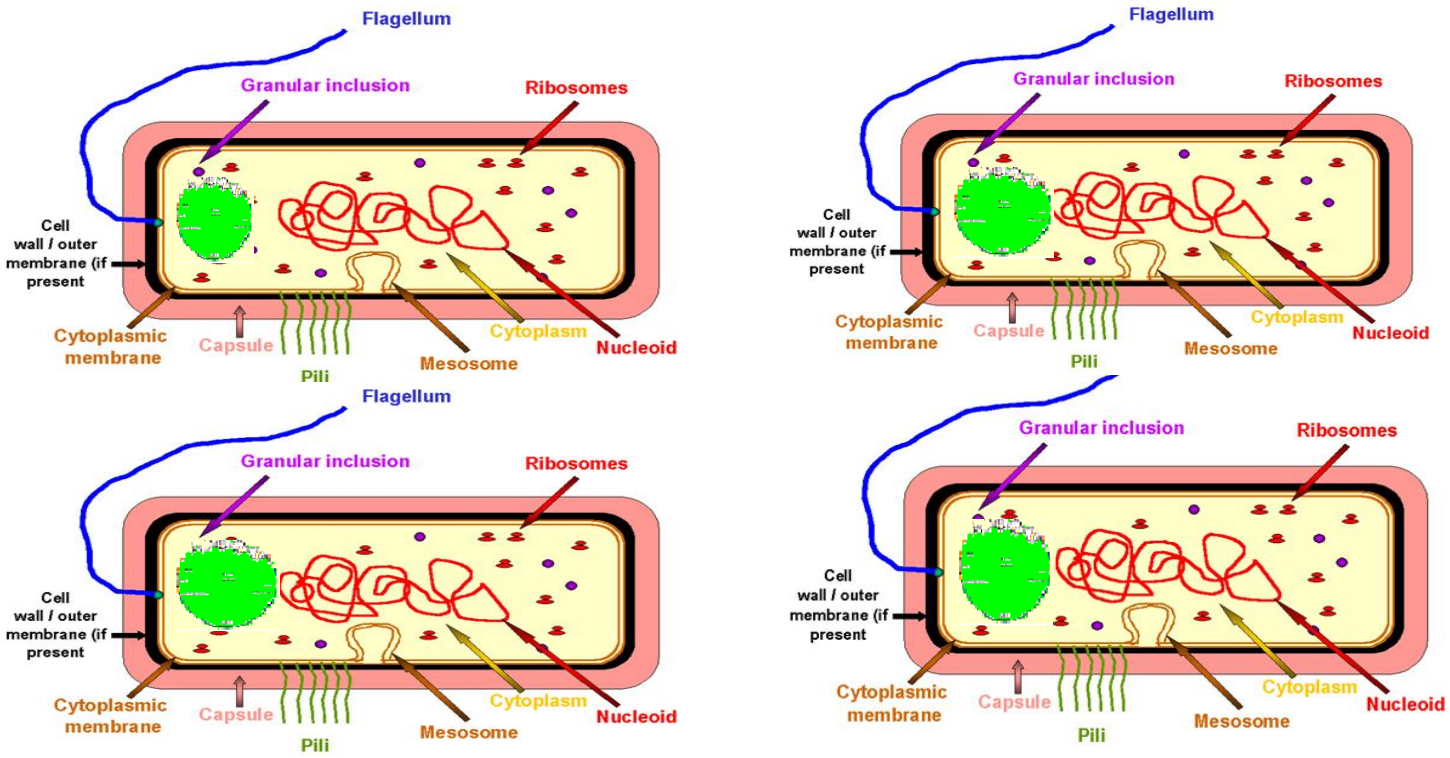

Gene of interest
Promoter
PolyA signal
Bacterial origin of replication
CpG CpG
Antibiotic resistance gene
Pathogen
Isolation of gene for antigenic protein
Cloning into a vaccine plasmid
Production by bacteria
Purification of plasmid
Administration

Transform into bacterial cell

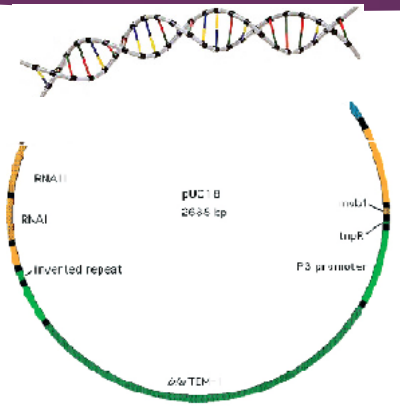


Bacterial cell

Plasmid DNA get Amplified



Plasmid DNA Purified



Ready to use

Advantages of the recombinant vaccine

- Produced more quickly
- In larger quantities
- Free from infectious viral particles.
- They can safely be given to immunosuppressed animal.
- They are less likely to induce side effects